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(54) Title: METHOD OF PREPARING A HEAT-TREATED PRODUCT

(57) Abstract: The formation of acrylamide during heat treatment in the production of a food product is reduced by treating the raw material with an enzyme before the heat treatment. The enzyme is capable of reacting on asparagine or glutamine (optionally substituted) as a substrate or is a laccase or a peroxidase.

METHOD OF PREPARING A HEAT-TREATED PRODUCT

FIELD OF THE INVENTION

The present invention relates to a method of preparing a heat-treated product with a low water content from raw material comprising carbohydrate, protein and water. It also relates to an asparaginase for use in the method

BACKGROUND OF THE INVENTION

E. Tabeke et al. (*J. Agric. Food Chem.*, 2002, *50*, 4998-5006) reported that acrylamide is formed during heating of starch-rich foods to high temperatures. The acrylamide formation has been ascribed to the Maillard reaction (D.S. Mottram et al., R.H. Stadtler et al., *Nature*, 419, 3 October 2002, 448-449).

WO 00/56762 discloses expressed sequence tags (EST) from *A. oryzae*.

Kim,K.-W.; Kamerud,J.Q.; Livingston,D.M.; Roon,R.J., (1988) Asparaginase II of Saccharomyces cerevisiae. Characterization of the ASP3 gene. J. Biol. Chem. 263:11948, discloses the peptide sequence of an extra-cellular asparaginase

SUMMARY OF THE INVENTION

According to the invention, the formation of acrylamide during heat treatment of raw material comprising carbohydrate, protein and water is reduced by treating the raw material with an enzyme before the heat treatment. Accordingly, the invention provides a method of preparing a heat-treated product, comprising the sequential steps of:

a) providing a raw material which comprises carbohydrate, protein and water
b) treating the raw material with an enzyme, and
c) heat treating to reach a final water content below 35 % by weight.

The enzyme is capable of reacting on asparagine or glutamine (optionally substituted) as a substrate or is a laccase or a peroxidase.

The invention also provides an asparaginase for use in the process and a polynucleotide encoding the asparaginase.

DETAILED DESCRIPTION OF THE INVENTION

Raw material and enzyme treatment

The raw material comprises carbohydrate, protein and water, typically in amounts of 10-90 % or 20-50 % carbohydrate of the total weight. The carbohydrate may consist mainly of starch, and it may include reducing sugars such as glucose, e.g. added as glucose syrup,

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honey or dry dextrose. The protein may include free amino acids such as asparagine and glutamine (optionally substituted).

The raw material may include tubers, potatoes, grains, oats, barley, corn (maize), wheat, nuts, fruits, dried fruit, bananas, sesame, rye and/or rice.

The raw material may be in the form of a dough comprising finely divided ingredients (e.g. flour) with water. The enzyme treatment may be done by mixing (kneading) the enzyme into the dough and optionally holding to let the enzyme act. The enzyme may be added in the form of an aqueous solution, a powder, a granulate or agglomerated powder. The dough may be formed into desired shapes, e.g. by sheeting, cutting and/or extrusion.

The raw material may also be in the form of intact vegetable pieces, e.g. slices or other pieces of potato, fruit or bananas, whole nuts, whole grains etc. The enzyme treatment may comprise immersing the vegetable pieces in an aqueous enzyme solution and optionally applying vacuum infusion. The intact pieces may optionally be blanched by immersion in hot water, e.g. at 70-100°C, either before or after the enzyme treatment.

The raw material may be grain intended for malting, e.g. malting barley or wheat. The enzyme treatment of the grain may be done before, during or after the malting (germination).

The raw material before heat treatment typically has a water content of 10-90 % by weight and is typically weakly acidic, e.g. having a pH of 5-7.

Heat treatment

The process of the invention involves a heat treatment at high temperature to reach a final water content (moisture content) in the product below 35 % by weight, typically 1-20 %, 1-10 % or 2-5 %. During the heat treatment, the temperature at the surface of the product may reach 110-220°C, e.g. 110-170°C or 120-160°C.

The heat treatment may involve, frying, particularly deep frying in tri- and/or di-glycerides (animal or vegetable oil or fat, optionally hydrogenated), e.g. at temperatures of 150-180°C. The heat treatment may also involve baking in hot air, e.g. at 160-310°C or 200-250°C for 2-10 minutes, or hot-plate heating. Further, the heat treatment may involve kilning of green malt.

Heat-treated product

The process of the invention may be used to produce a heat-treated product with low water content from raw material containing carbohydrate and protein, typically starchy food products fried or baked at high temperatures. The heat-treated product may be consumed directly as an edible product or may be used as an ingredient for further processing to prepare an edible or potable product.

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Examples of products to be consumed directly are potato products, potato chips (crisps), French fries, hash browns, roast potatoes, breakfast cereals, crisp bread, muesli, biscuits, crackers, snack products, tortilla chips, roasted nuts, rice crackers (Japanese "senbei"), wafers, waffles, hot cakes, and pancakes.

Malt (e.g. caramelized malt or so-called chocolate malt) is generally further processed by mashing and brewing to make beer.

Enzyme capable of reacting with asparagine or glutamine (optionally substituted) as a substrate

The enzyme may be capable of reacting with asparagine or glutamine which is optionally glycosylated or substituted with a peptide at the alpha-amino and/or the carboxyl position. The enzyme may be an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase or a peptidoglutaminase.

The glutaminase (EC 3.5.1.2) may be derived from *Escherichia coli*. The L-amino acid oxidase (EC 1.4.3.2) capable of reacting with asparagine or glutamine (optionally glycosylated) as a substrate may be derived from *Trichoderma harzianum* (WO 94/25574). The glycosylasparaginase (EC 3.5.1.26, aspartylglucosaminidase, N4-(N-acetyl-beta-glucosaminyl)-L-asparagine amidase) may be derived from *Flavobacterium meningosepticum*. The glycoamidase (peptide N-glycosidase, EC 3.5.1.52) may be derived from *Flavobacterium meningosepticum*. The peptidoglutaminase may be peptidoglutaminase I or II (EC 3.5.1.43, EC 3.5.1.44).

The enzyme is used in an amount which is effective to reduce the amount of acrylamide in the final product. The amount may be in the range 0.1-100 mg enzyme protein per kg dry matter, particularly 1-10 mg/kg. Asparaginase may be added in an amount of 10-100 units per kg dry matter where one unit will liberate 1 micromole of ammonia from L-asparagine per min at pH 8.6 at 37 °C

Asparaginase

The asparaginase (EC 3.5.1.1) may be derived from *Saccharomyces cerevisiae*, *Candia utilis*, *Escherichia coli*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Fusarium graminearum*, or *Penicillium citrinum*. It may have the amino acid sequence shown in SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378), 4, 6, 8, 10, 12 or 13 or a sequence which is at least 90 % (particularly at least 95 %) identical to one of these. It may be produced by use of the genetic information in SEQ ID NO: 1, 3, 5, 7, 9 or 11, e.g., as described in an example.

Whitehead Institute, MIT Center for Genome Research, Fungal Genome Initiative has published *A nidulans* release 1 and *F. graminearum* release 1 on the Internet at http://www-genome.wi.mit.edu/ftp/distribution/annotation/ under the *Aspergillus* Sequencing Project and

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the *Fusarium graminearum* Sequencing Project. Preliminary sequence data for *Aspergillus fumigatus* was published on The Institute for Genomic Research website at http://www-genome.wi.mit.edu/ftp/distribution/annotation/.

The inventors inserted the gene encoding the asparaginase from *A. oryzae* into *E. coli* and deposited the clone under the terms of the Budapest Treaty with the DSMZ - Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig. The deposit number was DSM 15960, deposited on 6 October 2003.

Alignment and identity

The enzyme and the nucleotide sequence of the invention may have homologies to the disclosed sequences of at least 90 % or at least 95 %, e.g. at least 98 %.

For purposes of the present invention, alignments of sequences and calculation of identity scores were done using a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap is -12 for proteins and -16 for DNA, while the penalty for additional residues in a gap is -2 for proteins and -4 for DNA. Alignment is from the FASTA package version v20u6 (W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", Methods in Enzymology, 183:63-98).

Laccase or peroxidase

The laccase (EC 1.10.3.2) may be of plant or microbial origin, e.g. from bacteria or fungi (including filamentous fungi and yeasts). Examples include laccase from *Aspergillus*, *Neurospora*, e.g., *N. crassa*, *Podospora*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, e.g., *T. villosa* and *T. versicolor*, *Rhizoctonia*, e.g., *R. solani*, *Coprinus*, e.g., *C. cinereus*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, e.g., *P. condelleana*, *Panaeolus*, e.g., *P. papilionaceus*, *Myceliophthora*, e.g., *M. thermophila*, *Schytalidium*, e.g., *S. thermophilum*, *Polyporus*, e.g., *P. pinsitus*, *Phlebia*, e.g., *P. radita*, or *Coriolus*, e.g., *C. hirsutus*.

The peroxidase (EC 1.11.1.7) may be from plants (e.g. horseradish or soybean peroxidase) or microorganisms such as fungi or bacteria, e.g. *Coprinus*, in particular *Coprinus cinereus* f. *microsporus* (IFO 8371), or *Coprinus macrorhizus*, *Pseudomonas*, e.g. *P. fluorescens* (NRRL B-11), *Streptoverticillium*, e.g. *S. verticillium* ssp. *verticillium* (IFO 13864), *Streptomyces*, e.g. *S. thermoviolaceus* (CBS 278.66), *Streptomyces*, e.g. *S. viridosporus* (ATCC 39115), *S. badius* (ATCC 39117), *S. phaeochromogenes* (NRRL B-3559), *Pseudomonas*, e.g. *P. pyrrocinia* (ATCC 15958), *Fusarium*, e.g. *F. oxysporum* (DSM 2672) and *Bacillus*, e.g. *B. stearothermophilus* (ATCC 12978).

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Oxidoreductase capable of reacting with a reducing sugar as a substrate

The method of the invention may comprise treating the raw material with an oxidoreductase capable of reacting with a reducing sugar as a substrate. The oxidoreductase may be an oxidase or dehydrogenase capable of reacting with a reducing sugar as a substrate such as glucose and maltose.

The oxidase may be a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which has a higher activity on maltose than on glucose. The glucose oxidase (EC 1.1.3.4) may be derived from *Aspergillus niger* e.g. having the amino acid sequence described in US 5094951. The hexose oxidase (EC 1.1.3.5) may be derived from algal species such as *Iridophycus flaccidum*, *Chondrus crispus* and *Euthora cristata*. The pyranose oxidase may be derived from *Basidiomycete* fungi, *Peniophora gigantean*, *Aphyllophorales*, *Phanerochaete chrysosporium*, *Polyporus pinsitus*, *Bierkandera adusta* or *Phlebiopsis gigantean*. The carbohydrate oxidase which has a higher activity on maltose than on glucose may be derived from *Microdochium* or *Acremonium*, e.g. from *M. nivale* (US 6165761), *A. strictum*, *A. fusidioides* or *A. potronii*.

The dehydrogenase may be glucose dehydrogenase (EC 1.1.1.47, EC 1.1.99.10), galactose dehydrogenase (EC 1.1.1.48), D-aldohexose dehydrogenase (EC 1.1.1.118, EC 1.1.1.119), cellobiose dehydrogenase (EC 1.1.5.1, e.g. from *Humicola insolens*), fructose dehydrogenase (EC 1.1.99.11, EC 1.1.1.124, EC 1.1.99.11), aldehyde dehydrogenase (EC 1.2.1.3, EC 1.2.1.4, EC 1.2.1.5). Another example is glucose-fructose oxidoreductase (EC 1.1.99.28).

The oxidoreductase is used in an amount which is effective to reduce the amount of acrylamide in the final product. For glucose oxidase, the amount may be in the range 50-20,000 (e.g. 100-10,000 or 1,000-5,000) GODU/kg dry matter in the raw material. One GODU is the amount of enzyme which forms 1 µmol of hydrogen peroxide per minute at 30°C, pH 5.6 (acetate buffer) with glucose 16.2 g/l (90 mM) as substrate using 20 min. incubation time. For other enzymes, the dosage may be found similarly by analyzing with the appropriate substrate.

EXAMPLES

Media

DAP2C-1

11g $MgSO_4 \cdot 7H_2O$

1g KH_2PO_4

2g Citric acid, monohydrate

30g maltodextrin

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6g $K_3PO_4 \cdot 3H_2O$

0.5g yeast extract

0.5ml trace metals solution

1ml Pluronic PE 6100 (BASF, Ludwigshafen, Germany)

Components are blended in one liter distilled water and portioned out to flasks, adding 250 mg CaCO3 to each 150ml portion.

The medium is sterilized in an autoclave. After cooling the following is added to 1 liter of medium:

23 ml 50% w/v $(NH_4)_2HPO_4$, filter sterilized

33 ml 20% lactic acid, filter sterilized

Trace metals solution

6.8g $ZnCl_2$

2.5g $CuSO_4 \cdot 5H_2O$

0.24g $NiCl_2 \cdot 6H_2O$

13.9g $FeSO_4 \cdot 7H_2O$

8.45g $MnSO_4 \cdot H_2O$

3g Citric acid, monohydrate

Components are blended in one liter distilled water.

Asparaginase activity assay

Stock solutions

50 mM Tris buffer, pH 8.6

189mM L-Asparagine solution

1.5 M Trichloroacetic Acid (TCA)

Nessler's reagent, Aldrich Stock No. 34,514-8 (Sigma-Aldrich, St. Louis, Mo. USA)

Asparaginase, Sigma Stock No. A4887 (Sigma-Aldrich, St. Louis, Mo. USA)

Assay

Enzyme reaction:

500 micro-l buffer

100 micro-l L-asparagine solution

350 micro-l water

are mixed and equilibrated to 37 °C.

100 micro-l of enzyme solution is added and the reactions are incubated at 37 °C for 30 minutes.

The reactions are stopped by placing on ice and adding 50 micro-l of 1.5M TCA.

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The samples are mixed and centrifuged for 2 minutes at 20,000 g

Measurement of free ammonium:

50 micro-l of the enzyme reaction is mixed with 100 micro-l of water and 50 micro-l of Nessler's reagent. The reaction is mixed and absorbance at 436nm is measured after 1 minute.

Standard:

The asparaginase stock (Sigma A4887) is diluted 0.2, 0.5, 1, 1.5, 2, and 2.5 U/ml.

Example 1: Expression of an asparaginase from *Aspergillus oryzae* in *Aspergillus oryzae*

Libraries of cDNA of mRNA from *Aspergillus oryzae* were generated, sequenced and stored in a computer database as described in WO 00/56762.

The peptide sequence of asparaginase II from *Saccharomyces cerevisiae* (Kim,K.-W.; Kamerud,J.Q.; Livingston,D.M.; Roon,R.J., (1988) Asparaginase II of Saccharomyces cerevisiae. Characterization of the ASP3 gene. J. Biol. Chem. 263:11948), was compared to translations of the *Aspergillus oryzae* partial cDNA sequences using the TFASTXY program, version 3.2t07 (Pearson et al, Genomics (1997) 46:24-36). One translated *A. oryzae* sequence was identified as having 52% identity to yeast asparaginase II through a 165 amino acid overlap. The complete sequence of the cDNA insert of the corresponding clone (deposited as DSM 15960) was determined and is presented as SEQ ID NO: 1, and the peptide translated from this sequence, AoASP, is presented as SEQ ID NO: 2. This sequence was used to design primers for PCR amplification of the AoASP encoding-gene from DSM 15960, with appropriate restriction sites added to the primer ends to facilitate sub-cloning of the PCR product (primers AoASP7 and AoASP8, SEQ ID NOS: 14 and 15). PCR amplification was performed using Extensor Hi-Fidelity PCR Master Mix (ABgene, Surrey, U.K.) following the manufacturer's instructions and using an annealing temperature of 55°C for the first 5 cycles and 65°C for an additional 30 cycles and an extension time of 1.5 minutes.

The PCR fragment was restricted with *BamHI* and *HindIII* and cloned into the *Aspergillus* expression vector pMStr57 using standard techniques. The expression vector pMStr57 contains the same elements as pCaHj483 (WO 98/00529), with minor modifications made to the *Aspergillus* NA2 promoter as described for the vector pMT2188 in WO 01/12794, and has sequences for selection and propogation in *E. coli*, and selection and expression in *Aspergillus*. Specifically, selection in *Aspergillus* is facilitated by the *amd*S gene of *Aspergillus nidulans*, which allows the use of acetamide as a sole nitrogen source. Expression in *Aspergillus* is mediated by a modified neutral amylase II (NA2) promoter from *Aspergillus niger* which is fused to the 5' leader sequence of the triose phosphate isomerase (tpi) encoding-gene from

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Aspergillus nidulans, and the terminator from the amyloglucosidase-encoding gene from *Aspergillus niger*. The asparaginase-encoding gene of the resulting *Aspergillus* expression construct, pMStr90, was sequenced and the sequence agreed completely with that determined previously for the insert of DSM 15960

The *Aspergillus oryzae* strain BECh2 (WO 00/39322) was transformed with pMStr90 using standard techniques (Christensen, T. et al., (1988), Biotechnology 6, 1419-1422). Transformants were cultured in DAP2C-1 medium shaken at 200 RPM at 30°C and expression of AoASP was monitored by SDS-PAGE and by measuring enzyme activity.

Example 2: Purification of Asparaginase

Culture broth from the preceding example was centrifuged (20000 x g, 20 min) and the supernatants were carefully decanted from the precipitates. The combined supernatants were filtered through a Seitz EKS plate in order to remove the rest of the *Aspergillus* host cells. The EKS filtrate was transferred to 10 mM Tris/HCl, pH 8 on a G25 sephadex column and applied to a Q sepharose HP column equilibrated in the same buffer. After washing the Q sepharose HP column extensively with the equilibration buffer, the asparaginase was eluted with a linear NaCl gradient (0 --> 0.5M) in the same buffer. Fractions from the column were analysed for asparaginase activity (using the pH 6.0 Universal buffer) and fractions with activity were pooled. Ammonium sulfate was added to the pool to 2.0M final concentration and the pool was applied to a Phenyl Toyopearl S column equilibrated in 20 mM succinic acid, 2.0M $(NH_4)_2SO_4$, pH 6.0. After washing the Phenyl column extensively with the equilibration buffer, the enzyme was eluted with a linear $(NH_4)_2SO_4$ gradient (2.0 --> 0M) in the same buffer. Fractions from the column were again analysed for asparaginase activity and active fractions were further analysed by SDS-PAGE. Fractions, which was judged only to contain the asparaginase, were pooled as the purified preparation and was used for further characterization. The purified asparaginase was heterogeneously glycosylated judged from the coomassie stained SDS-PAGE gel and in addition N-terminal sequencing of the preparation revealed that the preparation contained different asparaginase forms, as four different N-termini were found starting at amino acids A_{27}, S_{30}, G_{75} and A_{80} respectively of SEQ ID NO: 2. However, the N-terminal sequencing also indicated that the purified preparation was relatively pure as no other N-terminal sequences were found by the analysis.

Example 3: Properties of asparaginase

The purified asparaginase from the preceding example was used for characterization.

Asparaginase assay

A coupled enzyme assay was used. Asparaginase was incubated with asparagine and the liberated ammonia was determined with an Ammonia kit from Boehringer Mannheim

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(cat. no. 1 112 732) based on glutamate dehydrogenase and NADH oxidation to NAD^+ (can be measured as a decrease in A_{375}). Hence the decrease in absorbance at 375 nm was taken as a measure of asparaginase activity.

Asparagine substrate :	10mg/ml L-asparagine (Sigma A-7094) was dissolved in Universal buffers and pH was adjusted to the indicated pH-values with HCl or NaOH.
Temperature :	controlled
Universal buffers :	100 mM succinic acid, 100 mM HEPES, 100 mM CHES, 100 mM CABS, 1 mM $CaCl_2$, 150 mM KCl, 0.01% Triton X-100 adjusted to pH-values 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 with HCl or NaOH.
Stop reagent :	500 mM TCA (Trichloroacetic acid).
Assay buffer :	1.0M KH_2PO_4/NaOH, pH 7.5.
Ammonia reagent A :	1 NADH tablet + 1.0 ml Bottle 1 (contain 2-oxoglutarate (second substrate) and buffer) + 2.0 ml Assay buffer.
Ammonia reagent B :	40 micro-l Bottle 3 (contain glutamate dehydrogenase) + 1460 micro-l Assay buffer.

450 micro-l asparagine substrate was placed on ice in an Eppendorf tube. 50 micro-l asparaginase sample (diluted in 0.01% Triton X-100) was added. The assay was initiated by transferring the Eppendorf tube to an Eppendorf thermomixer, which was set to the assay temperature. The tube was incubated for 15 minutes on the Eppendorf thermomixer at its highest shaking rate (1400 rpm). The incubation was stopped by transferring the tube back to the ice bath and adding 500 micro-l Stop reagent. The tube was vortexed and centrifuged shortly in an icecold centrifuge to precipitate the proteins in the tube. The amount of ammonia liberated by the enzyme was measured by the following procedure: 20 micro-l supernatant was transferred to a microtiter plate, 200 micro-l Ammonia reagent A was added and A_{375} was read (A_{375}(initial)). Then 50 micro-l Ammonia reagent B was added and after 10 minutes at room temperature the plate was read again (A_{375}(final)). A_{375}(initial) – A_{375}(final) was a measure of asparaginase activity. A buffer blind was included in the assay (instead of enzyme) and the decrease in A_{375} in the buffer blind was subtracted from the enzyme samples.

pH-activity , pH-stability, and temperature-activity of asparaginase

The above asparaginase assay was used for obtaining the pH-activity profile, the pH-stability profile as well as the temperature-activity profile at pH 7.0. For the pH-stability profile the asparaginase was diluted 7x in the Universal buffers and incubated for 2 hours at 37°C.

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After incubation the asparaginase samples were transferred to neutral pH, before assay for residual activity, by dilution in the pH 7 Universal buffer.

The results for the: pH-activity profile at 37°C were as follows, relative to the residual activity at after 2 hours at pH 7.0 and 5°C :

pH	Asparaginase
2	0.00
3	0.01
4	0.10
5	0.53
6	0.95
7	1.00
8	0.66
9	0.22
10	0.08
11	0.00

The results for the pH-stability profile (residual activity after 2 hours at 37°C) were as follows:

pH	Asparaginase
2.0	0.00
3.0	0.00
4.0	1.06
5.0	1.08
6.0	1.09
7.0	1.09
8.0	0.92
9.0	0.00
10.0	0.00
11.0	0.00
12.0	0.00
	1.00

The results for the temperature activity profile (at pH 7.0) were as follows:

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Temp (°C)	Asparaginase
15	0.24
25	0.39
37	0.60
50	0.81
60	1.00
70	0.18

Other characteristics

The relative molecular weight as determined by SDS-PAGE was seen as a broad band (a smear) at M_r = 40-65 kDa.

N-terminal sequencing showed four different terminals, corresponding to residues 27-37, 30-40, 75-85 and 80-91 of SEQ ID NO: 2, respectively.

Example 3: Cloning of asparaginase from *Penicillium citrinum*

Penicillium citrinum was grown in MEX-1 medium (Medium B in WO 98/38288) in flasks shaken at 150RPM at 26°C for 3 and 4 days. Mycelium was harvested, a cDNA library constructed, and cDNAs encoding secreted peptides were selected and sequenced by the methods described in WO 03/044049. Comparison to known sequences by methods described in WO 03/044049 indicated that Penicillium sequence ZY132299 encoded an asparaginase. The complete sequence of the corresponding cDNA was determined and is presented as SEQ ID NO: 11, and the peptide translated from this sequence is presented as SEQ ID NO: 12.

Example 4: Effect of asparaginase on acrylamide content in potato chips

Asparaginase from *A. oryzae* having the amino acid sequence shown in SEQ ID NO: 2 was prepared and purified as in Examples 1-2 and added at various dosages to potato chips made from 40 g of water, 52.2 g of dehydrated potato flakes, 5.8 g of potato starch and 2 g of salt.

The flour and dry ingredients were mixed for 30 sec. The salt and enzyme were dissolved in the water, and the solution was adjusted to 30°C The solution was added to the flour. The dough was further mixed for 15 min. The mixed dough was placed in a closed plastic bag and allowed to rest for 15 min at room temperature.

The dough was then initially compressed for 60 sec in a dough press.

The dough was sheeted and folded in a noodle roller machine until an approx. 5-10 mm dough is obtained. The dough was then rolled around a rolling pin and allowed to rest for

BNSDOCID: <WO_____2004032648A1_I_>

30 min in a plastic bag at room temperature. The dough was sheeted further to a final sheet thickness of approx 1.2 mm.

The sheet was cut into squares of approx 3 x 5 cm.

The sheets were placed in a frying basket, placed in an oil bath and fried for 45 sec at 180° C. The noodle basket was held at a 45° angle until the oil stopped dripping. The products were removed from the basket and left to cool on dry absorbent paper.

The potato chips were homogenized and analyzed for acrylamide. The results were as follows:

Asparaginase dosage U/kg potato dry matter	Acrylamide Micro-g per kg
0	5,200
100	4,600
500	3,100
1000	1,200
2000	150

The results demonstrate that the asparaginase treatment is effective to reduce the acrylamide content in potato chips, that the acrylamide reduction is clearly dosage dependent, and that the acrylamide content can be reduced to a very low level.

Example 5: Effect of various enzymes on acrylamide content in potato chips

Potato chips were made as follows with addition of enzyme systems which are capable of reacting on asparagine, as indicated below.

Recipe:

Tap water	40 g
Potato flakes dehydrated	52.2 g
Potato starch	5.8 g
Salt	2 g

Dough Procedure:

The potato flakes and potato starch are mixed for 30 sec in a mixer at speed 5. Salt and enzyme are dissolved in the water. The solution is adjusted to 30°C +/- 1°C. Stop mixer, add all of the salt/enzyme solution to flour. The dough is further mixed for 15 min.

Place mixed dough in plastic bag, close bag and allow the dough to rest for 15 min at room temperature.

The dough is then initially compressed for 60 sec in a dough press.

BNSDOCID: <WO_____2004032648A1_I_>

The dough is sheeted and folded in a noodle roller machine until an approx. 5-10 mm dough is obtained. The dough is then rolled around a rolling pin and the dough is allowed to rest for 30 min in a plastic bag at room temperature. The dough is sheeted further to a final sheet thickness of approx 1.2 mm.

Cut the sheet into squares of approx 3 x 5 cm.

Sheets are placed in a frying basket, placed in the oil bath and fried for 60 sec at 180°C. Hold the noodle basket at a 45° angle and let the product drain until oil stops dripping. Remove the products from the basket and leave them to cool on dry absorbent paper.

The results from acrylamide analysis were as follows:

Enzyme	Enzyme dosage per kg of potato dry matter	Acrylamide Micro-g per kg
None (control)	0	4,100
Asparaginase from *Erwinia Chrysanthemi* A-2925	1000 U/kg	150
Glutaminase (product of Daiwa)	50 mg enzyme protein/kg	1,800
Amino acid oxidase from *Trichoderma harzianum* described in WO 9425574.	50 mg enzyme protein/kg	1,300
Laccase from *Myceliophthora thermophila* + peroxidase from *Coprinus*	5000 LAMU/kg + 75 mg enzyme protein/kg	2,000

The results demonstrate that all the tested enzyme systems are effective in reducing the acrylamide content of potato chips.

BNSDOCID: <WO_____2004032648A1_I_>

PCT

Original (for **SUBMISSION**) - printed on 10.10.2003 09:39:26 AM

0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.07.2003)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	10347-WO

1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	4
1-2	line	5-7
1-3	Identification of Deposit	
1-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	06 October 2003 (06.10.2003)
1-3-4	Accession Number	DSMZ 15960
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	YES
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

BNSDOCID: <WO_____2004032648A1_I_>

DSMZ
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshojvej 36
DK-2880 Bagsvaerd

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Novozymes A/S Address: Krogshojvej 36 DK-2880 Bagsvaerd	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15960 Date of the deposit or the transfer[1]: 2003-10-06

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2003-10-06
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weils Date: 2003-10-13

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 12/2001

BNSDOCID: <WO_____2004032648A1_I_>

DSMZ
Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshojvej 36
DK-2880 Bagsvaerd

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the DEPOSITOR:	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY:
NN049697	DSM 15960

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I. above was accompanied by:

( ) a scientific description
(x) a proposed taxonomic designation

(Mark with a cross where applicable).

III. RECEIPT AND ACCEPTANCE

This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-10-06 (Date of the original deposit)[1].

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).

V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2003-10-13

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 12/2001

BNSDOCID: <WO_____2004032648A1_I_>

CLAIMS

1. A method of preparing a heat-treated product, comprising the sequential steps of:
   a) providing a raw material which comprises carbohydrate, protein and water
   b) treating the raw material with an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, a laccase or a peroxidase, and
   c) heat treating to reach a final water content below 35 % by weight.

2. The method of the preceding claim wherein the enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate is an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase (peptide N-glycosidase) or a peptidoglutaminase.

3. The method of the preceding claim wherein the asparaginase has an amino acid sequence which is at least 90 % identical to SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378), 4, 6, 8, 10, 12 or 13.

4. The method of any preceding claim which further comprises treating the raw material with an oxidoreductase capable of reacting with a reducing sugar as a substrate.

5. The method of the preceding claim wherein the oxidoreductase capable of reacting with a reducing sugar as a substrate is a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which has a higher activity on maltose than on glucose.

6. The method of any preceding claim wherein the raw material is in the form of a dough and the enzyme treatment comprises mixing the enzyme into the dough and optionally holding.

7. The method of any preceding claim wherein the raw material comprises intact vegetable pieces and the enzyme treatment comprises immersing the potato pieces in an aqueous solution of the enzyme.

8. The method of any preceding claim wherein the raw material comprises a potato product.

BNSDOCID: <WO_____2004032648A1_I_>

9. A polypeptide having asparaginase activity and having an amino acid sequence which is at least 90 % identical with SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378) or SEQ ID NO: 12.

10. A polynucleotide encoding the polypeptide of the preceding claim.

11. A polynucleotide which encodes an asparaginase and which comprises a nucleotide sequence which is at least 90 % identical to the coding sequences of SEQ ID NO: 1 or 11.

BNSDOCID: <WO_____2004032648A1_I_>

10347-WO-ST25
SEQUENCE LISTING

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10347-WO-ST25

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            180                 185                 190

Thr Lys Glu Ala Arg Asn Arg Gly Ala Met Val Val Met Asn Asp Arg
        195                 200                 205

Ile Ala Ser Ala Tyr Tyr Val Ser Lys Thr Asn Ala Asn Thr Met Asp
    210                 215                 220

Thr Phe Lys Ala Val Glu Met Gly Tyr Leu Gly Ala Ile Ile Ser Asn
225                 230                 235                 240

Thr Pro Phe Phe Tyr Tyr Pro Ala Val Gln Pro Ser Gly Lys Thr Thr
                245                 250                 255

Val Asp Val Ser Asn Val Thr Ser Ile Pro Arg Val Asp Ile Leu Tyr
            260                 265                 270

Ser Phe Gln Asp Met Thr Asn Asp Thr Leu Tyr Ser Ser Ile Glu Asn
        275                 280                 285

Gly Ala Lys Gly Val Val Ile Ala Gly Ser Gly Ala Gly Ser Val Asp
    290                 295                 300

Thr Ala Phe Ser Thr Ala Ile Asp Asp Ile Ile Ser Asn Gln Gly Val
305                 310                 315                 320

Pro Ile Val Gln Ser Thr Arg Thr Gly Asn Gly Glu Val Pro Tyr Ser
                325                 330                 335

Ala Glu Gly Gly Ile Ser Ser Gly Phe Leu Asn Pro Ala Lys Ser Arg
            340                 345                 350

Ile Leu Leu Gly Leu Leu Leu Ala Gln Gly Gly Lys Gly Thr Glu Glu
        355                 360                 365

Ile Arg Ala Val Phe Gly Lys Val Ala Val
    370                 375
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<210> 5
<211> 1330
<212> DNA
<213> Aspergillus fumigatus

<220>
<221> CDS
<222> (93)..(978)

<220>
<221> CDS
<222> (1056)..(1291)

<400> 5
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BNSDOCID: <WO_____2004032648A1_I_>

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agcagagcag ttcgcctcgt cagatcgcaa ag atg acc aaa ctc agc ttc aaa      113
                                    Met Thr Lys Leu Ser Phe Lys
                                    1               5

atc atc aca ctc gcg gct atg ata gcc gtt ggg aat gcc tct ccg ttt      161
Ile Ile Thr Leu Ala Ala Met Ile Ala Val Gly Asn Ala Ser Pro Phe
        10                  15                  20

gtc tac ccc cga gca acc agc cca aac agt aca tat gtc ttc acc aac      209
Val Tyr Pro Arg Ala Thr Ser Pro Asn Ser Thr Tyr Val Phe Thr Asn
    25                  30                  35

tcg cat ggc ttg aac ttc acc cag atg aac acg acg ctc cct aat gtc      257
Ser His Gly Leu Asn Phe Thr Gln Met Asn Thr Thr Leu Pro Asn Val
40                  45                  50                  55

acc atc ctc gca acc ggc ggt acc att gcc ggc tcc agc aac gac aac      305
Thr Ile Leu Ala Thr Gly Gly Thr Ile Ala Gly Ser Ser Asn Asp Asn
                60                  65                  70

acc gcc aca aca ggc tac acg gcc ggc gcg atc ggc atc cag cag ctc      353
Thr Ala Thr Thr Gly Tyr Thr Ala Gly Ala Ile Gly Ile Gln Gln Leu
            75                  80                  85

atg gat gcc gtc cct gag atg cta gac gtt gct aac gtg gcc ggc atc      401
Met Asp Ala Val Pro Glu Met Leu Asp Val Ala Asn Val Ala Gly Ile
        90                  95                  100

cag gtc gcc aat gtc ggc agc ccc gac gtg acg tct tcc ctt ctg ctc      449
Gln Val Ala Asn Val Gly Ser Pro Asp Val Thr Ser Ser Leu Leu Leu
    105                 110                 115

cac atg gcc agg acc atc aac gag gtc gtc tgc gac gac ccc acc atg      497
His Met Ala Arg Thr Ile Asn Glu Val Val Cys Asp Asp Pro Thr Met
120                 125                 130                 135

agc ggc gcc gtc atc acg cac ggc acc gac acg ctc gag gag acg gcc      545
Ser Gly Ala Val Ile Thr His Gly Thr Asp Thr Leu Glu Glu Thr Ala
                140                 145                 150

ttc ttc ctc gac gct aca gtc aac tgc ggc aag ccc atc gtc gtc gtc      593
Phe Phe Leu Asp Ala Thr Val Asn Cys Gly Lys Pro Ile Val Val Val
            155                 160                 165

ggc gcc atg cgg ccc gca acc gcc atc tcc gcc gac ggc ccg ttc aac      641
Gly Ala Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Phe Asn
        170                 175                 180

ctc ctc cag gcc gtg acc gtc gcc gcg cac ccc act gcg cgc aac cgt      689
Leu Leu Gln Ala Val Thr Val Ala Ala His Pro Thr Ala Arg Asn Arg
    185                 190                 195

ggt gcg ctg gtc gtc atg aac gac cgc att gtg tcc gcg tac tac gtc      737
Gly Ala Leu Val Val Met Asn Asp Arg Ile Val Ser Ala Tyr Tyr Val
200                 205                 210                 215

tcc aag aca aac gcc aac acc atg gac acc ttc aag gcc gtc gag atg      785
Ser Lys Thr Asn Ala Asn Thr Met Asp Thr Phe Lys Ala Val Glu Met
                220                 225                 230

ggc aac ctc ggc gcc atc atc tcc aac aag ccg tac ttc ttt tac ccg      833
Gly Asn Leu Gly Ala Ile Ile Ser Asn Lys Pro Tyr Phe Phe Tyr Pro
            235                 240                 245

ccc gtc atg ccc acc ggt aag acc act ttc gac gtg cgc aac gtc gcc      881
Pro Val Met Pro Thr Gly Lys Thr Thr Phe Asp Val Arg Asn Val Ala
        250                 255                 260
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BNSDOCID: <WO_____2004032648A1_I_>

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tcc atc ccc aga gtc gac atc ctc tac tcg tac cag gat atg caa aac      929
Ser Ile Pro Arg Val Asp Ile Leu Tyr Ser Tyr Gln Asp Met Gln Asn
    265                 270                 275

gat acg ctc tac gac gcc gtc gac aac ggc gcg aaa ggc atc gtc gta a    978
Asp Thr Leu Tyr Asp Ala Val Asp Asn Gly Ala Lys Gly Ile Val Val
280                 285                 290                 295

gtccagcccc tttctaaagc cctcaccgga tcaaccgctg aaattgaacc taatccagat   1038

cgccggctcc ggcgcag ga  agc gtc tca agt ggc tac tac gat gcc atc      1087
                     Arg Ser Val Ser Ser Gly Tyr Tyr Asp Ala Ile
                                     300                 305

gac gac atc gca tcc acg cac tcc ctc cct gtc gtc ctc agc act cgc     1135
Asp Asp Ile Ala Ser Thr His Ser Leu Pro Val Val Leu Ser Thr Arg
            310                 315                 320

acc ggc aac ggc gaa gtc gcc atc aca gac agc gag acc aca att gag     1183
Thr Gly Asn Gly Glu Val Ala Ile Thr Asp Ser Glu Thr Thr Ile Glu
        325                 330                 335

agc ggc ttc ctg aac ccg cag aaa gcg cgc atc ctg ctc ggt ctg ctg     1231
Ser Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Leu Leu Gly Leu Leu
    340                 345                 350

ctt gct gag gat aag gga ttc aag gag atc aaa gag gcg ttc gcg aag     1279
Leu Ala Glu Asp Lys Gly Phe Lys Glu Ile Lys Glu Ala Phe Ala Lys
355                 360                 365                 370

aac ggg gtt gct tgattatgtc cttccttgtt ttgggtggca tttgtggtt          1330
Asn Gly Val Ala
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<210> 6
<211> 374
<212> PRT
<213> Aspergillus fumigatus

<400> 6

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Met Thr Lys Leu Ser Phe Lys Ile Ile Thr Leu Ala Ala Met Ile Ala
1               5                   10                  15

Val Gly Asn Ala Ser Pro Phe Val Tyr Pro Arg Ala Thr Ser Pro Asn
            20                  25                  30

Ser Thr Tyr Val Phe Thr Asn Ser His Gly Leu Asn Phe Thr Gln Met
        35                  40                  45

Asn Thr Thr Leu Pro Asn Val Thr Ile Leu Ala Thr Gly Gly Thr Ile
    50                  55                  60

Ala Gly Ser Ser Asn Asp Asn Thr Ala Thr Thr Gly Tyr Thr Ala Gly
65                  70                  75                  80

Ala Ile Gly Ile Gln Gln Leu Met Asp Ala Val Pro Glu Met Leu Asp
                85                  90                  95

Val Ala Asn Val Ala Gly Ile Gln Val Ala Asn Val Gly Ser Pro Asp
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BNSDOCID: <WO_____2004032648A1_I_>

100 105 110

Val Thr Ser Ser Leu Leu Leu His Met Ala Arg Thr Ile Asn Glu Val
115 120 125

Val Cys Asp Asp Pro Thr Met Ser Gly Ala Val Ile Thr His Gly Thr
130 135 140

Asp Thr Leu Glu Glu Thr Ala Phe Phe Leu Asp Ala Thr Val Asn Cys
145 150 155 160

Gly Lys Pro Ile Val Val Val Gly Ala Met Arg Pro Ala Thr Ala Ile
165 170 175

Ser Ala Asp Gly Pro Phe Asn Leu Leu Gln Ala Val Thr Val Ala Ala
180 185 190

His Pro Thr Ala Arg Asn Arg Gly Ala Leu Val Val Met Asn Asp Arg
195 200 205

Ile Val Ser Ala Tyr Tyr Val Ser Lys Thr Asn Ala Asn Thr Met Asp
210 215 220

Thr Phe Lys Ala Val Glu Met Gly Asn Leu Gly Ala Ile Ile Ser Asn
225 230 235 240

Lys Pro Tyr Phe Phe Tyr Pro Pro Val Met Pro Thr Gly Lys Thr Thr
245 250 255

Phe Asp Val Arg Asn Val Ala Ser Ile Pro Arg Val Asp Ile Leu Tyr
260 265 270

Ser Tyr Gln Asp Met Gln Asn Asp Thr Leu Tyr Asp Ala Val Asp Asn
275 280 285

Gly Ala Lys Gly Ile Val Val Arg Ser Val Ser Ser Gly Tyr Tyr Asp
290 295 300

Ala Ile Asp Asp Ile Ala Ser Thr His Ser Leu Pro Val Val Leu Ser
305 310 315 320

Thr Arg Thr Gly Asn Gly Glu Val Ala Ile Thr Asp Ser Glu Thr Thr
325 330 335

Ile Glu Ser Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Leu Leu Gly
340 345 350

Leu Leu Leu Ala Glu Asp Lys Gly Phe Lys Glu Ile Lys Glu Ala Phe
355 360 365

Ala Lys Asn Gly Val Ala

BNSDOCID: <WO_____2004032648A1_I_>

370

<210> 7
<211> 1260
<212> DNA
<213> Fusarium graminearum

<220>
<221> CDS
<222> (105)..(1217)

<400> 7
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ctgcgatcgc agaggaggag cagtcttttt cttctcgttc tttacctccc ccctcctcta      60

tctccagtct ctccaagtgt tgtgccctct tgtgttagcc cagc atg tcg ccc tct      116
                                                 Met Ser Pro Ser
                                                 1

ttc cac tcc cta ctc gct atc gca acc ctt gca ggc tca gct gcc ctt      164
Phe His Ser Leu Leu Ala Ile Ala Thr Leu Ala Gly Ser Ala Ala Leu
5                   10                  15                  20

gca tcc ccg atc ccg gag cca gaa aca ccg cag ctt atc ccc cgg gct      212
Ala Ser Pro Ile Pro Glu Pro Glu Thr Pro Gln Leu Ile Pro Arg Ala
                25                  30                  35

gtt ggt gac ttt gag tgc ttc aac gct agt ctt ccc aac atc acc atc      260
Val Gly Asp Phe Glu Cys Phe Asn Ala Ser Leu Pro Asn Ile Thr Ile
            40                  45                  50

ttc gcg act ggt ggt acc atc gct ggt tct gct ggt tct gcc gat cag      308
Phe Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Gly Ser Ala Asp Gln
        55                  60                  65

act acg ggt tac cag gct ggt gca ttg ggt atc caa gcg ttg atc gac      356
Thr Thr Gly Tyr Gln Ala Gly Ala Leu Gly Ile Gln Ala Leu Ile Asp
    70                  75                  80

gct gtc ccg caa ctc tgc aac gtc tcc aac gtc agg ggt gtg cag atc      404
Ala Val Pro Gln Leu Cys Asn Val Ser Asn Val Arg Gly Val Gln Ile
85                  90                  95                  100

gcc aac gtt gat agc ggc gat gta aac tct act atc ctg acc act ttg      452
Ala Asn Val Asp Ser Gly Asp Val Asn Ser Thr Ile Leu Thr Thr Leu
                105                 110                 115

gcg cat cgc atc cag act gat ctt gac aac cct cac atc caa ggt gtt      500
Ala His Arg Ile Gln Thr Asp Leu Asp Asn Pro His Ile Gln Gly Val
            120                 125                 130

gtc gtc acc cat ggc aca gac act ctc gag gag tct tca ttt ttc ctc      548
Val Val Thr His Gly Thr Asp Thr Leu Glu Glu Ser Ser Phe Phe Leu
        135                 140                 145

gat ctc act gtc caa agt gaa aag cct gtt gtt atg gtt gga tcc atg      596
Asp Leu Thr Val Gln Ser Glu Lys Pro Val Val Met Val Gly Ser Met
    150                 155                 160

cgt cct gcc act gcc atc agc gct gat ggt ccc atc aac ctc ctg tct      644
Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Ile Asn Leu Leu Ser
165                 170                 175                 180

gct gtt cga ttg gca ggt agc aag agt gcc aag ggt cgc ggt aca atg      692
Ala Val Arg Leu Ala Gly Ser Lys Ser Ala Lys Gly Arg Gly Thr Met
                185                 190                 195
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BNSDOCID: <WO_____2004032648A1_I_>

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att gta ctc aac gac aag atc gct tct gca cgc tac acc gtt aaa tcc        740
Ile Val Leu Asn Asp Lys Ile Ala Ser Ala Arg Tyr Thr Val Lys Ser
            200                 205                 210

cac gcc aat gct gtc cag act ttc att gcc gaa gat caa ggt tat ctt        788
His Ala Asn Ala Val Gln Thr Phe Ile Ala Glu Asp Gln Gly Tyr Leu
        215                 220                 225

ggt gcc ttt gaa aac att cag ccc gtc ttc tgg tac cct gct agt cga        836
Gly Ala Phe Glu Asn Ile Gln Pro Val Phe Trp Tyr Pro Ala Ser Arg
    230                 235                 240

cca cta ggt cac cac tat ttc aac att agt gct agc tca cct aag aag        884
Pro Leu Gly His His Tyr Phe Asn Ile Ser Ala Ser Ser Pro Lys Lys
245                 250                 255                 260

gct ctt cct cag gtt gac gtt ttg tac ggc cac caa gaa gcg gac ccc        932
Ala Leu Pro Gln Val Asp Val Leu Tyr Gly His Gln Glu Ala Asp Pro
                265                 270                 275

gag ctt ttc caa gct gct gtc gat agc ggc gcc cag ggc att gtt ctc        980
Glu Leu Phe Gln Ala Ala Val Asp Ser Gly Ala Gln Gly Ile Val Leu
            280                 285                 290

gct ggt ctt ggc gct gga ggc tgg cct gac gaa gct gct gat gag atc       1028
Ala Gly Leu Gly Ala Gly Gly Trp Pro Asp Glu Ala Ala Asp Glu Ile
        295                 300                 305

aag aag gtc ttg aac gag act aac att cct gtt gtt gtc agc cgt cgt       1076
Lys Lys Val Leu Asn Glu Thr Asn Ile Pro Val Val Val Ser Arg Arg
    310                 315                 320

act gct tgg ggt tac gtt gga gag agg cct ttc ggt atc ggt gct ggg       1124
Thr Ala Trp Gly Tyr Val Gly Glu Arg Pro Phe Gly Ile Gly Ala Gly
325                 330                 335                 340

tac ttg aac cct tcc aag gcc aga atc caa ctg caa ctt gcg ctt gag       1172
Tyr Leu Asn Pro Ser Lys Ala Arg Ile Gln Leu Gln Leu Ala Leu Glu
                345                 350                 355

aag aag ctt tct gtg gag gag atc caa gac ata ttc gag tat gtt           1217
Lys Lys Leu Ser Val Glu Glu Ile Gln Asp Ile Phe Glu Tyr Val
            360                 365                 370

tgattggaag aggattttga aatgaatcaa tgatatatga tta                      1260


<210>  8
<211>  371
<212>  PRT
<213>  Fusarium graminearum

<400>  8

Met Ser Pro Ser Phe His Ser Leu Leu Ala Ile Ala Thr Leu Ala Gly
1               5                   10                  15

Ser Ala Ala Leu Ala Ser Pro Ile Pro Glu Pro Glu Thr Pro Gln Leu
            20                  25                  30

Ile Pro Arg Ala Val Gly Asp Phe Glu Cys Phe Asn Ala Ser Leu Pro
        35                  40                  45

Asn Ile Thr Ile Phe Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Gly
    50                  55                  60
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BNSDOCID: <WO_____2004032648A1_I_>

Ser Ala Asp Gln Thr Thr Gly Tyr Gln Ala Gly Ala Leu Gly Ile Gln
65 70 75 80

Ala Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ser Asn Val Arg
85 90 95

Gly Val Gln Ile Ala Asn Val Asp Ser Gly Asp Val Asn Ser Thr Ile
100 105 110

Leu Thr Thr Leu Ala His Arg Ile Gln Thr Asp Leu Asp Asn Pro His
115 120 125

Ile Gln Gly Val Val Val Thr His Gly Thr Asp Thr Leu Glu Glu Ser
130 135 140

Ser Phe Phe Leu Asp Leu Thr Val Gln Ser Glu Lys Pro Val Val Met
145 150 155 160

Val Gly Ser Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Ile
165 170 175

Asn Leu Leu Ser Ala Val Arg Leu Ala Gly Ser Lys Ser Ala Lys Gly
180 185 190

Arg Gly Thr Met Ile Val Leu Asn Asp Lys Ile Ala Ser Ala Arg Tyr
195 200 205

Thr Val Lys Ser His Ala Asn Ala Val Gln Thr Phe Ile Ala Glu Asp
210 215 220

Gln Gly Tyr Leu Gly Ala Phe Glu Asn Ile Gln Pro Val Phe Trp Tyr
225 230 235 240

Pro Ala Ser Arg Pro Leu Gly His His Tyr Phe Asn Ile Ser Ala Ser
245 250 255

Ser Pro Lys Lys Ala Leu Pro Gln Val Asp Val Leu Tyr Gly His Gln
260 265 270

Glu Ala Asp Pro Glu Leu Phe Gln Ala Ala Val Asp Ser Gly Ala Gln
275 280 285

Gly Ile Val Leu Ala Gly Leu Gly Ala Gly Gly Trp Pro Asp Glu Ala
290 295 300

Ala Asp Glu Ile Lys Lys Val Leu Asn Glu Thr Asn Ile Pro Val Val
305 310 315 320

Val Ser Arg Arg Thr Ala Trp Gly Tyr Val Gly Glu Arg Pro Phe Gly
325 330 335

BNSDOCID: <WO_____2004032648A1_I_>

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Ile Gly Ala Gly Tyr Leu Asn Pro Ser Lys Ala Arg Ile Gln Leu Gln
            340                 345                 350

Leu Ala Leu Glu Lys Lys Leu Ser Val Glu Glu Ile Gln Asp Ile Phe
        355                 360                 365

Glu Tyr Val
    370


<210>  9
<211>  1470
<212>  DNA
<213>  Fusarium graminearum


<220>
<221>  CDS
<222>  (77)..(1429)

<400>  9
aggacaagcg tccatgaagc ataactacgc tacattgcct ttagctacag ttgatctata      60

gatatcagtc tacatc atg atg ccc agc gtc aga aga ttt cac ggc cag act     112
                  Met Met Pro Ser Val Arg Arg Phe His Gly Gln Thr
                  1               5                   10

atg gtc gcc gcc gct cct tct att tgc tca ggg cct gca gca tcg tcc       160
Met Val Ala Ala Ala Pro Ser Ile Cys Ser Gly Pro Ala Ala Ser Ser
        15                  20                  25

acc atc aag atg gct tca tcg tca gct tcg tgg acg act tat ctg tgg       208
Thr Ile Lys Met Ala Ser Ser Ser Ala Ser Trp Thr Thr Tyr Leu Trp
    30                  35                  40

cgg ctt atc cta gct gtg ctg gct cct tca acg gcc ctg ctg cct ttt       256
Arg Leu Ile Leu Ala Val Leu Ala Pro Ser Thr Ala Leu Leu Pro Phe
45                  50                  55                  60

ggt gcg tgg gtt gtt tcg gtc tgg gga tct cct gtc ctc gac cta cac       304
Gly Ala Trp Val Val Ser Val Trp Gly Ser Pro Val Leu Asp Leu His
                65                  70                  75

gtc caa cct cac ttc tcg gtt caa caa aaa gcg cca ata cag acg ggc       352
Val Gln Pro His Phe Ser Val Gln Gln Lys Ala Pro Ile Gln Thr Gly
            80                  85                  90

atc cct ttc gaa att tcg acc acc tca gga ttc aac tgc ttc aat ccc       400
Ile Pro Phe Glu Ile Ser Thr Thr Ser Gly Phe Asn Cys Phe Asn Pro
        95                  100                 105

aat ctt ccc aac gtc act att tat gcc acc gga ggt act att gct ggc       448
Asn Leu Pro Asn Val Thr Ile Tyr Ala Thr Gly Gly Thr Ile Ala Gly
    110                 115                 120

tcc gca agc tcg gct gat cag acc acg gga tac cgg tca gct gcg tta       496
Ser Ala Ser Ser Ala Asp Gln Thr Thr Gly Tyr Arg Ser Ala Ala Leu
125                 130                 135                 140

gga gtt gat tct ctc att gat gca gta ccc caa ttg tgc aat gta gcc       544
Gly Val Asp Ser Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ala
                145                 150                 155

aat gtg aga ggt gtc cag ttt gcc aac acg gac agc ata gac atg agc       592
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Asn Val Arg Gly Val Gln Phe Ala Asn Thr Asp Ser Ile Asp Met Ser
160 165 170

tcg gcc atg ttg agg act ttg gcg aag cag atc cag aat gat ctg gac 640
Ser Ala Met Leu Arg Thr Leu Ala Lys Gln Ile Gln Asn Asp Leu Asp
175 180 185

agt ccg ttt act caa ggc gca gtt gtg acg cac gga act gat act ctg 688
Ser Pro Phe Thr Gln Gly Ala Val Val Thr His Gly Thr Asp Thr Leu
190 195 200

gat gaa tct gcc ttc ttt ctg gat ctt act atc cag agc gac aag ccc 736
Asp Glu Ser Ala Phe Phe Leu Asp Leu Thr Ile Gln Ser Asp Lys Pro
205 210 215 220

gtg gtc gtg aca ggc tca atg cgc ccg gca act gct atc agc gca gat 784
Val Val Val Thr Gly Ser Met Arg Pro Ala Thr Ala Ile Ser Ala Asp
225 230 235

gga cca atg aat ctt ttg tca tcg gtg aca ttg gca gca gca gcg agt 832
Gly Pro Met Asn Leu Leu Ser Ser Val Thr Leu Ala Ala Ala Ala Ser
240 245 250

gct cga ggc aga gga gtg atg att gcc atg aat gat cgc att gga tct 880
Ala Arg Gly Arg Gly Val Met Ile Ala Met Asn Asp Arg Ile Gly Ser
255 260 265

gct cgt ttt acg acc aaa gtc aac gcc aac cat ttg gac gcc ttc caa 928
Ala Arg Phe Thr Thr Lys Val Asn Ala Asn His Leu Asp Ala Phe Gln
270 275 280

gcc cct gac agt ggc atg ctg gga aca ttc gtc aac gtt cag cca gtg 976
Ala Pro Asp Ser Gly Met Leu Gly Thr Phe Val Asn Val Gln Pro Val
285 290 295 300

ttt ttc tat ccg cca tca cga cct ctt ggc cac cgt cat ttt gat ctg 1024
Phe Phe Tyr Pro Pro Ser Arg Pro Leu Gly His Arg His Phe Asp Leu
305 310 315

cgg ccc atc acc aac aac ggc cgc cgg ttc gga cgc tct aca gcc ccc 1072
Arg Pro Ile Thr Asn Asn Gly Arg Arg Phe Gly Arg Ser Thr Ala Pro
320 325 330

gga gca gga tca tca gca cta ccc cag gtg gac gtg ctc tac gct tac 1120
Gly Ala Gly Ser Ser Ala Leu Pro Gln Val Asp Val Leu Tyr Ala Tyr
335 340 345

cag gag ctc agc gtg ggc atg ttc cag gcg gcc atc gac ctt gga gcg 1168
Gln Glu Leu Ser Val Gly Met Phe Gln Ala Ala Ile Asp Leu Gly Ala
350 355 360

cag ggc atc gtt cta gcg gga atg ggc gct gga ttc tgg acg tcc aaa 1216
Gln Gly Ile Val Leu Ala Gly Met Gly Ala Gly Phe Trp Thr Ser Lys
365 370 375 380

ggt acc gag gag att cgg cgt atc gtc cac gag acc gat att ccc gtg 1264
Gly Thr Glu Glu Ile Arg Arg Ile Val His Glu Thr Asp Ile Pro Val
385 390 395

ata gtg agc cga aga ccg gaa ggc ggc ttc gtc gga cca tgt gag gca 1312
Ile Val Ser Arg Arg Pro Glu Gly Gly Phe Val Gly Pro Cys Glu Ala
400 405 410

gga atc ggc gcg ggc ttt ttg aat ccg caa aag gcg agg atc cag ctc 1360
Gly Ile Gly Ala Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Gln Leu
415 420 425

caa ctg gcc ctg gag acc aag atg gac aat gat gcc atc aaa gcc ctg 1408

BNSDOCID: <WO_____2004032648A1_I_>

Gln Leu Ala Leu Glu Thr Lys Met Asp Asn Asp Ala Ile Lys Ala Leu
430 435 440

ttt gag cat tcg gga gtg cac taaagggaca aaaaagatcg aggttacagc 1459
Phe Glu His Ser Gly Val His
445 450

agcaacacca c 1470

<210> 10
<211> 451
<212> PRT
<213> Fusarium graminearum

<400> 10

Met Met Pro Ser Val Arg Arg Phe His Gly Gln Thr Met Val Ala Ala
1 5 10 15

Ala Pro Ser Ile Cys Ser Gly Pro Ala Ala Ser Ser Thr Ile Lys Met
20 25 30

Ala Ser Ser Ser Ala Ser Trp Thr Thr Tyr Leu Trp Arg Leu Ile Leu
35 40 45

Ala Val Leu Ala Pro Ser Thr Ala Leu Leu Pro Phe Gly Ala Trp Val
50 55 60

Val Ser Val Trp Gly Ser Pro Val Leu Asp Leu His Val Gln Pro His
65 70 75 80

Phe Ser Val Gln Gln Lys Ala Pro Ile Gln Thr Gly Ile Pro Phe Glu
85 90 95

Ile Ser Thr Thr Ser Gly Phe Asn Cys Phe Asn Pro Asn Leu Pro Asn
100 105 110

Val Thr Ile Tyr Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Ser Ser
115 120 125

Ala Asp Gln Thr Thr Gly Tyr Arg Ser Ala Ala Leu Gly Val Asp Ser
130 135 140

Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ala Asn Val Arg Gly
145 150 155 160

Val Gln Phe Ala Asn Thr Asp Ser Ile Asp Met Ser Ser Ala Met Leu
165 170 175

Arg Thr Leu Ala Lys Gln Ile Gln Asn Asp Leu Asp Ser Pro Phe Thr
180 185 190

Gln Gly Ala Val Val Thr His Gly Thr Asp Thr Leu Asp Glu Ser Ala
195 200 205

BNSDOCID: <WO_____2004032648A1_I_>

Phe Phe Leu Asp Leu Thr Ile Gln Ser Asp Lys Pro Val Val Val Thr
210 215 220

Gly Ser Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Met Asn
225 230 235 240

Leu Leu Ser Ser Val Thr Leu Ala Ala Ala Ala Ser Ala Arg Gly Arg
245 250 255

Gly Val Met Ile Ala Met Asn Asp Arg Ile Gly Ser Ala Arg Phe Thr
260 265 270

Thr Lys Val Asn Ala Asn His Leu Asp Ala Phe Gln Ala Pro Asp Ser
275 280 285

Gly Met Leu Gly Thr Phe Val Asn Val Gln Pro Val Phe Phe Tyr Pro
290 295 300

Pro Ser Arg Pro Leu Gly His Arg His Phe Asp Leu Arg Pro Ile Thr
305 310 315 320

Asn Asn Gly Arg Arg Phe Gly Arg Ser Thr Ala Pro Gly Ala Gly Ser
325 330 335

Ser Ala Leu Pro Gln Val Asp Val Leu Tyr Ala Tyr Gln Glu Leu Ser
340 345 350

Val Gly Met Phe Gln Ala Ala Ile Asp Leu Gly Ala Gln Gly Ile Val
355 360 365

Leu Ala Gly Met Gly Ala Gly Phe Trp Thr Ser Lys Gly Thr Glu Glu
370 375 380

Ile Arg Arg Ile Val His Glu Thr Asp Ile Pro Val Ile Val Ser Arg
385 390 395 400

Arg Pro Glu Gly Gly Phe Val Gly Pro Cys Glu Ala Gly Ile Gly Ala
405 410 415

Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Gln Leu Gln Leu Ala Leu
420 425 430

Glu Thr Lys Met Asp Asn Asp Ala Ile Lys Ala Leu Phe Glu His Ser
435 440 445

Gly Val His
450

<210> 11
<211> 1236
<212> DNA

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<213>  Penicillium citrinum

<220>
<221>  CDS
<222>  (16)..(1152)

<400>  11
acatattgaa acaat atg aga ctt cta ttt aat act ctg gct gtc tca gca      51
                 Met Arg Leu Leu Phe Asn Thr Leu Ala Val Ser Ala
                 1               5                   10

cta gct gct acg agt tat gcc tct ccc atc att cat tcc cgg gcc tcc       99
Leu Ala Ala Thr Ser Tyr Ala Ser Pro Ile Ile His Ser Arg Ala Ser
        15                  20                  25

aac acg tcc tat acc aac tct aat ggg ctg aaa ttt aac cat ttc gac      147
Asn Thr Ser Tyr Thr Asn Ser Asn Gly Leu Lys Phe Asn His Phe Asp
    30                  35                  40

gct tct ctt cca aat gtg act ttg ctg gca act ggt gga act att gcc      195
Ala Ser Leu Pro Asn Val Thr Leu Leu Ala Thr Gly Gly Thr Ile Ala
45                  50                  55                  60

ggt aca agc gat gac aag act gct acg gca gga tat gaa tcc ggg gct      243
Gly Thr Ser Asp Asp Lys Thr Ala Thr Ala Gly Tyr Glu Ser Gly Ala
                65                  70                  75

tta ggg ata aat aag att ctt tcc ggc atc cca gaa gtt tat gac att      291
Leu Gly Ile Asn Lys Ile Leu Ser Gly Ile Pro Glu Val Tyr Asp Ile
            80                  85                  90

gcc aac gtc aat gcg gta cag ttt gac aat gtc aac agc ggc gat gtc      339
Ala Asn Val Asn Ala Val Gln Phe Asp Asn Val Asn Ser Gly Asp Val
        95                  100                 105

tct yca tct ctc tta ctg aac atg aca cat acc ctt caa aag acc gtt      387
Ser Xaa Ser Leu Leu Leu Asn Met Thr His Thr Leu Gln Lys Thr Val
    110                 115                 120

tgt gat gac cct acg ata tct ggc gcc gtc atc acc cat ggc acc gat      435
Cys Asp Asp Pro Thr Ile Ser Gly Ala Val Ile Thr His Gly Thr Asp
125                 130                 135                 140

acc ctg gaa gaa tct gcc ttc ttc atc gat gca aca gtc aac tgc ggc      483
Thr Leu Glu Glu Ser Ala Phe Phe Ile Asp Ala Thr Val Asn Cys Gly
                145                 150                 155

aag ccg att gtg ttc gtt ggc tca atg cga cct tcc acc gca atc tct      531
Lys Pro Ile Val Phe Val Gly Ser Met Arg Pro Ser Thr Ala Ile Ser
            160                 165                 170

gcc gat ggc cct atg aat ttg ctc cag gga gtg act gtg gcc gct gac      579
Ala Asp Gly Pro Met Asn Leu Leu Gln Gly Val Thr Val Ala Ala Asp
        175                 180                 185

aaa cag gct aag aac cgc gga gca cta gtc gtg ctg aat gac cgc att      627
Lys Gln Ala Lys Asn Arg Gly Ala Leu Val Val Leu Asn Asp Arg Ile
    190                 195                 200

gtc tct gct ttc ttc gct aca aag aca aat gcg aat aca atg gac act      675
Val Ser Ala Phe Phe Ala Thr Lys Thr Asn Ala Asn Thr Met Asp Thr
205                 210                 215                 220

ttc aag gct tat gaa caa ggc agt ctt ggc atg att gtt tca aac aag      723
Phe Lys Ala Tyr Glu Gln Gly Ser Leu Gly Met Ile Val Ser Asn Lys
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ccc tac ttc tat tat ccg gca gtc gag cca aac gcg aag cac gtt gtt      771
Pro Tyr Phe Tyr Tyr Pro Ala Val Glu Pro Asn Ala Lys His Val Val
            240                 245                 250

cat ctt gac gac gtg gat gcg atc ccc cgt gtg gat att ctc tac gct      819
His Leu Asp Asp Val Asp Ala Ile Pro Arg Val Asp Ile Leu Tyr Ala
        255                 260                 265

tac gag gac atg cat agc gac tcc ctt cac agt gct atc aaa aat gga      867
Tyr Glu Asp Met His Ser Asp Ser Leu His Ser Ala Ile Lys Asn Gly
    270                 275                 280

gcc aag ggc atc gtg gtc gcc ggc gag ggc gca ggt ggt atc tcc acg      915
Ala Lys Gly Ile Val Val Ala Gly Glu Gly Ala Gly Gly Ile Ser Thr
285                 290                 295                 300

gac ttt agt gat acc atc gat gag att gca tcg aag cat cag att ccc      963
Asp Phe Ser Asp Thr Ile Asp Glu Ile Ala Ser Lys His Gln Ile Pro
                305                 310                 315

att atc ctg agc cac aga acc gtg aac gga gaa gtt cct act gct gat     1011
Ile Ile Leu Ser His Arg Thr Val Asn Gly Glu Val Pro Thr Ala Asp
            320                 325                 330

att acg ggt gat agc gcg aag act cgc att gca agt ggc atg tat aac     1059
Ile Thr Gly Asp Ser Ala Lys Thr Arg Ile Ala Ser Gly Met Tyr Asn
        335                 340                 345

ccc cag cag gcg cgc gtc ttg ctt gga cta ttg ctc gca gaa ggc aag     1107
Pro Gln Gln Ala Arg Val Leu Leu Gly Leu Leu Leu Ala Glu Gly Lys
    350                 355                 360

aag ttt gag gat att cga act atc ttc gga aaa gct act gtt gcc         1152
Lys Phe Glu Asp Ile Arg Thr Ile Phe Gly Lys Ala Thr Val Ala
365                 370                 375

tagacccacg tcatatatta tgcccatact tgggaacact tgaaactgat agactaaatt   1212

aattatattg tcgtttgttg ccgg                                          1236
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<213> Penicillium citrinum

<220>
<221> misc_feature
<222> (110)..(110)
<223> The 'Xaa' at location 110 stands for Pro, or Ser.

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Thr Asn Ser Asn Gly Leu Lys Phe Asn His Phe Asp Ala Ser Leu Pro
        35                  40                  45

Asn Val Thr Leu Leu Ala Thr Gly Gly Thr Ile Ala Gly Thr Ser Asp
    50                  55                  60
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BNSDOCID: <WO_____2004032648A1_I_>

Asp Lys Thr Ala Thr Ala Gly Tyr Glu Ser Gly Ala Leu Gly Ile Asn
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Lys Ile Leu Ser Gly Ile Pro Glu Val Tyr Asp Ile Ala Asn Val Asn
85 90 95

Ala Val Gln Phe Asp Asn Val Asn Ser Gly Asp Val Ser Xaa Ser Leu
100 105 110

Leu Leu Asn Met Thr His Thr Leu Gln Lys Thr Val Cys Asp Asp Pro
115 120 125

Thr Ile Ser Gly Ala Val Ile Thr His Gly Thr Asp Thr Leu Glu Glu
130 135 140

Ser Ala Phe Phe Ile Asp Ala Thr Val Asn Cys Gly Lys Pro Ile Val
145 150 155 160

Phe Val Gly Ser Met Arg Pro Ser Thr Ala Ile Ser Ala Asp Gly Pro
165 170 175

Met Asn Leu Leu Gln Gly Val Thr Val Ala Ala Asp Lys Gln Ala Lys
180 185 190

Asn Arg Gly Ala Leu Val Val Leu Asn Asp Arg Ile Val Ser Ala Phe
195 200 205

Phe Ala Thr Lys Thr Asn Ala Asn Thr Met Asp Thr Phe Lys Ala Tyr
210 215 220

Glu Gln Gly Ser Leu Gly Met Ile Val Ser Asn Lys Pro Tyr Phe Tyr
225 230 235 240

Tyr Pro Ala Val Glu Pro Asn Ala Lys His Val Val His Leu Asp Asp
245 250 255

Val Asp Ala Ile Pro Arg Val Asp Ile Leu Tyr Ala Tyr Glu Asp Met
260 265 270

His Ser Asp Ser Leu His Ser Ala Ile Lys Asn Gly Ala Lys Gly Ile
275 280 285

Val Val Ala Gly Glu Gly Ala Gly Gly Ile Ser Thr Asp Phe Ser Asp
290 295 300

Thr Ile Asp Glu Ile Ala Ser Lys His Gln Ile Pro Ile Ile Leu Ser
305 310 315 320

His Arg Thr Val Asn Gly Glu Val Pro Thr Ala Asp Ile Thr Gly Asp
325 330 335

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Ser Ala Lys Thr Arg Ile Ala Ser Gly Met Tyr Asn Pro Gln Gln Ala
340 345 350

Arg Val Leu Leu Gly Leu Leu Leu Ala Glu Gly Lys Lys Phe Glu Asp
355 360 365

Ile Arg Thr Ile Phe Gly Lys Ala Thr Val Ala
370 375

<210> 13
<211> 362
<212> PRT
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<400> 13

Met Arg Ser Leu Asn Thr Leu Leu Leu Ser Leu Phe Val Ala Met Ser
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Pro Ser Ile Lys Ile Phe Gly Thr Gly Gly Thr Ile Ala Ser Lys Gly
35 40 45

Ser Thr Ser Ala Thr Thr Ala Gly Tyr Ser Val Gly Leu Thr Val Asn
50 55 60

Asp Leu Ile Glu Ala Val Pro Ser Leu Ala Glu Lys Ala Asn Leu Asp
65 70 75 80

Tyr Leu Gln Val Ser Asn Val Gly Ser Asn Ser Leu Asn Tyr Thr His
85 90 95

Leu Ile Pro Leu Tyr His Gly Ile Ser Glu Ala Leu Ala Ser Asp Asp
100 105 110

Tyr Ala Gly Ala Val Val Thr His Gly Thr Asp Thr Met Glu Glu Thr
115 120 125

Ala Phe Phe Leu Asp Leu Thr Ile Asn Ser Glu Lys Pro Val Cys Ile
130 135 140

Ala Gly Ala Met Arg Pro Ala Thr Ala Thr Ser Ala Asp Gly Pro Met
145 150 155 160

Asn Leu Tyr Gln Ala Val Ser Ile Ala Ala Ser Glu Lys Ser Leu Gly
165 170 175

Arg Gly Thr Met Ile Thr Leu Asn Asp Arg Ile Ala Ser Gly Phe Trp
180 185 190

BNSDOCID: <WO_____2004032648A1_I_>

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Thr Thr Lys Met Asn Ala Asn Ser Leu Asp Thr Phe Arg Ala Asp Glu
        195                 200                 205

Gln Gly Tyr Leu Gly Tyr Phe Ser Asn Asp Asp Val Glu Phe Tyr Tyr
    210                 215                 220

Pro Pro Val Lys Pro Asn Gly Trp Gln Phe Phe Asp Ile Ser Asn Leu
225                 230                 235                 240

Thr Asp Pro Ser Glu Ile Pro Glu Val Ile Ile Leu Tyr Ser Tyr Gln
                245                 250                 255

Gly Leu Asn Pro Glu Leu Ile Val Lys Ala Val Lys Asp Leu Gly Ala
            260                 265                 270

Lys Gly Ile Val Leu Ala Gly Ser Gly Ala Gly Ser Trp Thr Ala Thr
        275                 280                 285

Gly Ser Ile Val Asn Glu Gln Leu Tyr Glu Glu Tyr Gly Ile Pro Ile
    290                 295                 300

Val His Ser Arg Arg Thr Ala Asp Gly Thr Val Pro Pro Asp Asp Ala
305                 310                 315                 320

Pro Glu Tyr Ala Ile Gly Ser Gly Tyr Leu Asn Pro Gln Lys Ser Arg
                325                 330                 335

Ile Leu Leu Gln Leu Cys Leu Tyr Ser Gly Tyr Gly Met Asp Gln Ile
            340                 345                 350

Arg Ser Val Phe Ser Gly Val Tyr Gly Gly
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<213> Artificial

<220>
<223> Primer AoASP8

<400> 15
atcaagcttc tattatccat cccatcca 28

BNSDOCID: <WO_____2004032648A1_I_>

INTERNATIONAL SEARCH REPORT

Internatic pplication No
PCT/DK 03/00684

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/03 A21D8/04 A23L1/217 A23L1/105 C12N9/82
C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A21D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/28729 A (NOVONORDISK AS ; SI JOAN QI (DK)) 22 December 1994 (1994-12-22) claims 1,12,13,16,17 page 9, paragraph 1 -----	1,4-6
X	WO 94/28728 A (NOVONORDISK AS ; SI JOAN QI (DK)) 22 December 1994 (1994-12-22) claims 1,5,11 page 8, paragraph 1 -----	1,4-6
X	US 2002/004085 A1 (OLSEN HANS SEJR ET AL) 10 January 2002 (2002-01-10) the whole document -----	1,6-8

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 January 2004	09/02/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Vuillamy, V

Form PCT/ISA/210 (second sheet) (July 1992)

BNSDOCID: <WO_____2004032648A1_I_>

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/00029 A (NOVONORDISK AS ;WAGNER PETER (DK); NIELSEN PER MUNK (DK)) 8 January 1998 (1998-01-08) page 9, line 22 - line 31 page 6, line 29 - page 7, line 9	1-5,7,9
X	WO 02/30207 A (BUDOLFSEN GITTE ;NOVOZYMES AS (DK); CHRISTIANSEN LUISE (DK)) 18 April 2002 (2002-04-18) claims; example 1	1,2,6
X	US 6 039 982 A (SI JOAN QI ET AL) 21 March 2000 (2000-03-21) column 4, line 24 - line 39 column 6, paragraph 2 - paragraph '0003! claims	1,2,4-6
X	DATABASE WPI Section Ch, Week 199815 Derwent Publications Ltd., London, GB; Class D11, AN 1998-162469 XP002235162 & JP 10 028516 A (KAO CORP) 3 February 1998 (1998-02-03) abstract	1,2,4-6
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 05, 30 May 1997 (1997-05-30) & JP 09 009862 A (CALPIS FOOD IND CO LTD:THE;AJINOMOTO CO INC), 14 January 1997 (1997-01-14) abstract	1
A	"Brief Communications" NATURE, vol. 419, 3 October 2002 (2002-10-03), pages 448-449, XP002235161 USA cited in the application the whole document	1
A	BIEKMAN E S A: "TOEPASSING VAN ENZYMEN BIJ DE VERWERKING VAN AARDAPPELEN TOT CONSUMPTIEPRODUKTEN" VOEDINGSMIDDELEN TECHNOLOGIE, NOORDERVLIET B.V. ZEIST, NL, vol. 22, no. 20, 12 October 1989 (1989-10-12), pages 51-53, XP000069625 ISSN: 0042-7934 the whole document	1,4,5,7,8

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International Application No
PCT/DK 03/00684

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/39828 A (DANISCO ;SOE JOERN BORCH (DK); PETERSEN LARS WEXOEE (US)) 23 May 2002 (2002-05-23) claims; example 11	1
A	K.W. KIM: "Asparaginase II of Saccharomyces cerevisiae" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 263, no. 24, 1988, pages 11948-11953, XP002266820 USA cited in the application the whole document	3

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Information on patent family members

International Application No
PCT/DK 03/00684

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9428729	A	22-12-1994	WO	9428729	A1	22-12-1994
			EP	0701403	A1	20-03-1996
WO 9428728	A	22-12-1994	AT	188343	T	15-01-2000
			DE	69422516	D1	10-02-2000
			DE	69422516	T2	06-07-2000
			WO	9428728	A1	22-12-1994
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			GR	3032941	T3	31-07-2000
			PT	702519	T	30-06-2000
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			WO	0178524	A2	25-10-2001
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			AU	3166997	A	21-01-1998
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			DE	69707881	T2	18-07-2002
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			IN	183759	A1	01-04-2000
			JP	2000513231	T	10-10-2000
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			AU	701661	B2	04-02-1999
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			CA	2236476	A1	19-06-1997
			CN	1203515	A ,B	30-12-1998
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			DE	69623644	T2	28-05-2003
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			DK	865241	T3	23-12-2002
			EP	0865241	A1	23-09-1998
			IN	183745	A1	01-04-2000
			JP	2000509245	T	25-07-2000
JP 10028516	A	03-02-1998	NONE			
JP 09009862	A	14-01-1997	NONE			
WO 0239828	A	23-05-2002	AU	1942202	A	27-05-2002
			CA	2427914	A1	23-05-2002
			EP	1341422	A2	10-09-2003
			WO	0239828	A2	23-05-2002
			US	2002114864	A1	22-08-2002